A Ratiometric Molecular Rotor for Measuring Membrane Viscosity

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Viscosity

- Classical Mechanical Definition
  - Resistance to an isotropic flow
  - Determines fluid strain rate
- Membrane Biology Definition
  - Commonly defined as the ease of movement of a theoretical particle through the lipid membrane
  - Governs many physiological and metabolic functions of the cell
  - Determines mobility of intermembrane proteins

Membrane Viscosity

- Changes in membrane viscosity are often indicative of intracellular conditions
  - Carrier mediated Transport (1)
  - Membrane bound receptors (1)
- Variety of cells exhibit increases in membrane viscosity
  - Diabetic Pancreatic Cells (2,3)
  - Malignant Cells (4)
  - Alzheimer's Disease (5)

1-D.H. Rohrbach, R. Timpl, Molecular and Cellular Aspects of Basement Membranes, Academic Press, San Diego, CA, 1993.
2-O. Nadiv, Y. Zick et al., Elevated protein tyrosine activity and increased membrane viscosity associated with impaired activation of the insulin receptor kinase in old rate. Biochem. J. 298
3- W. Osterode, C. Holler, F. Ulberth, Nutritional antioxidants, red cell membrane fluidity and blood viscosity in type 1 (insulin-dependent) diabetes mellitus, Diabe. Med. 10 (1996) 1044-1050
4-M.Shinitzky, Membrane fluidity in malignancy. Adversative and recuperative, Biochem. Biophys. Acta 738 (1984) 251-261
5-K.Scheur, A. Maras, W.F. Gattaz, N. Cairns, H. Forstl, W.E. Muller, Cortical NMDA receptor properties and membrane fluidity are altered in Alzheimer's disease, Dementia 7 (1996) 210-214

Atherosclerosis and Membrane Viscosity

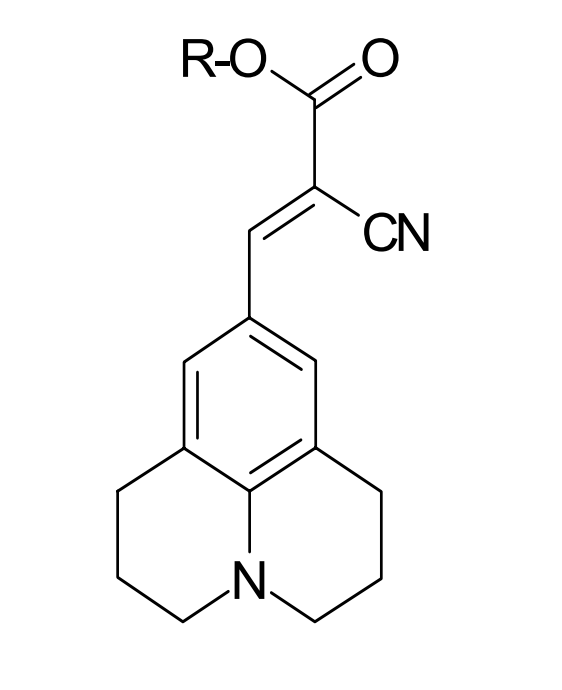
- Increased membrane viscosity inhibits EDRF/NO growth factor
- Vasosensitive mediator
- Cholesterol modulates EDRF production through viscosity regulation
- High cholesterol prohibits proper protein function
- Found to be reversible

Current Methods

- FRAP
  - Focused laser beam photobleaches area on membrane
    - Induced cross-linking from photo-oxidation
    - May damage functional proteins
    - Error is a function of laser beam radius
- Fluorescent Anisotropy
  - Internal rotation changes polarization plane
    - Rapid photobleaching
    - Filter absorption of signal

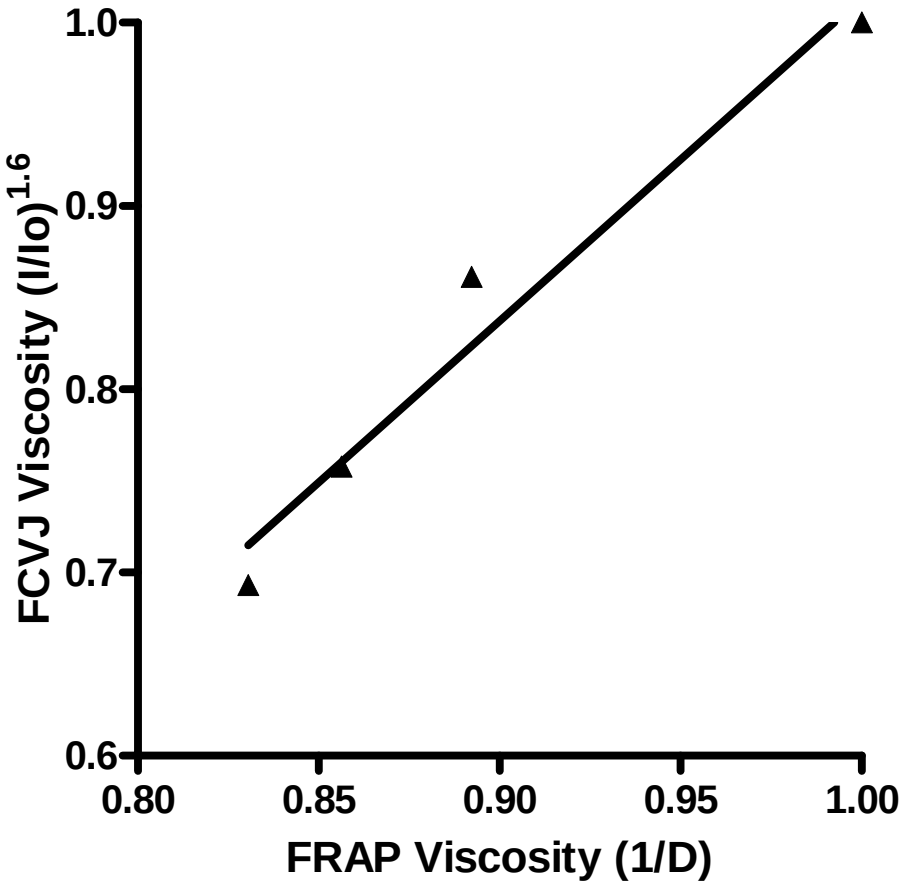
Molecular Rotors

- T.I.C.T.- Twisted Intermolecular Charge Transfer Molecules
- Forster-Hoffman equation: $\log\Phi = C + X\log\eta$
  - Φ=Quantum Yield
  - η=Viscosity dependent parameter



Previous Work

- Use the Molecular Rotor FCVJ to detect membrane viscosity change
- Incorporated the molecular rotor FCVJ into lipid membrane and changed the local viscosity
- Very good agreement with calculated FRAP viscosity

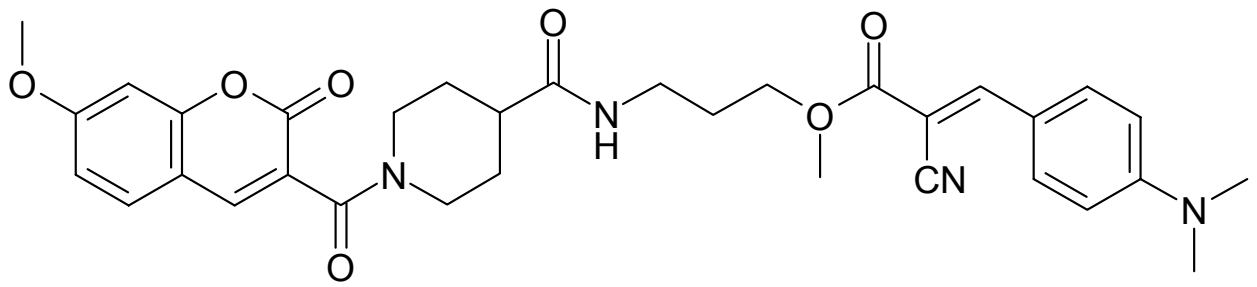

1.0
0.9
0.8
0.7
0.6
FCVJ Viscosity (I/Io)^1.6
0.80
0.85
0.90
0.95
1.00
FRAP Viscosity (1/D)

R^2=.96

Ratiometric Viscosity Sensitivity

- Molecular rotor attached to a reference dye
- Able to account for concentration effects
- Exceptional performance in viscosity gradient solutions

The Ratiometric Dye



Experimental Objectives

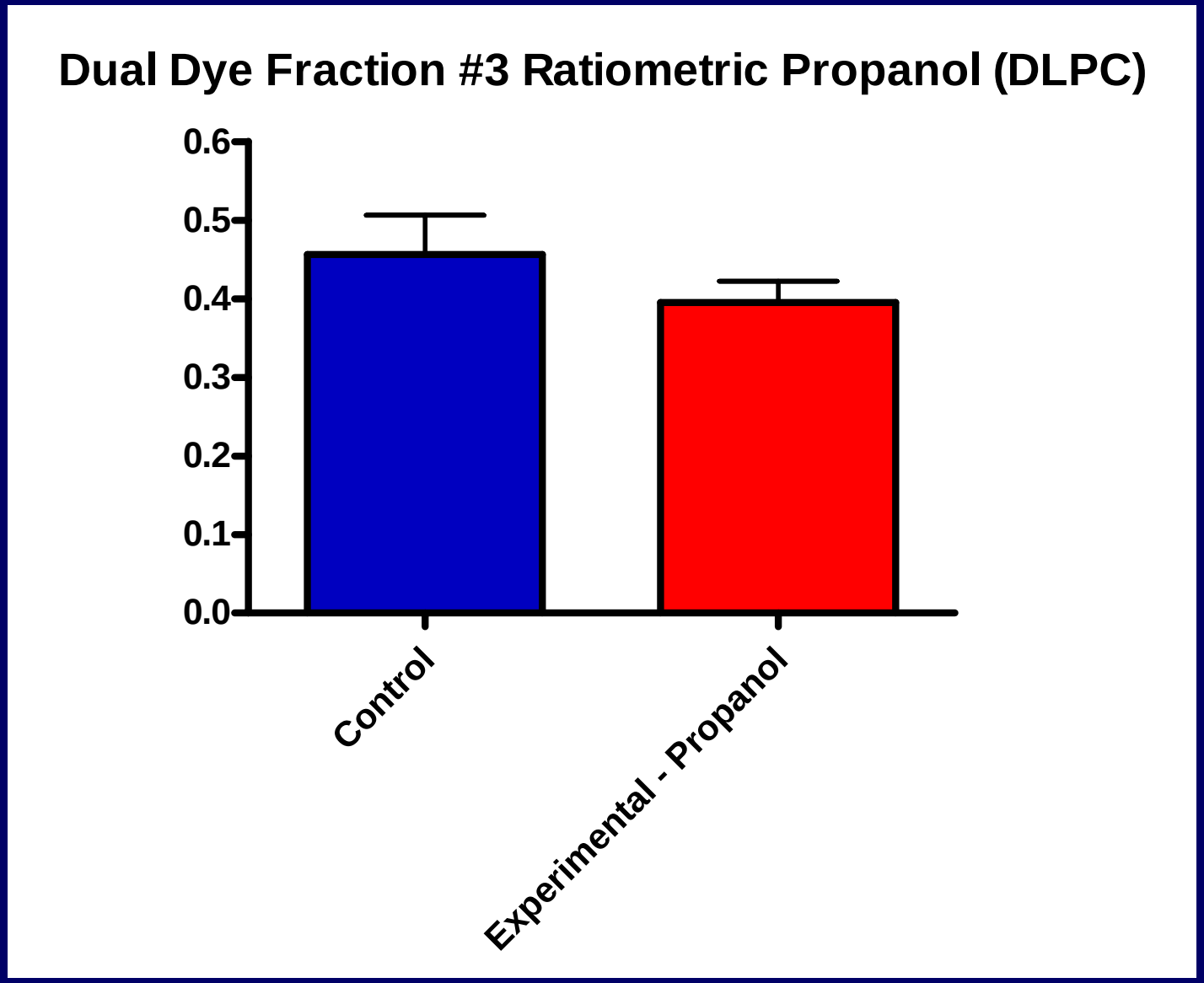
- Form liposomes to act as mammalian cell model
- Establish sensitivity through known previous experiments
- Show consistent viscosity conditions with increasing dye concentration
- Exhibit reduction of error compared to previous work

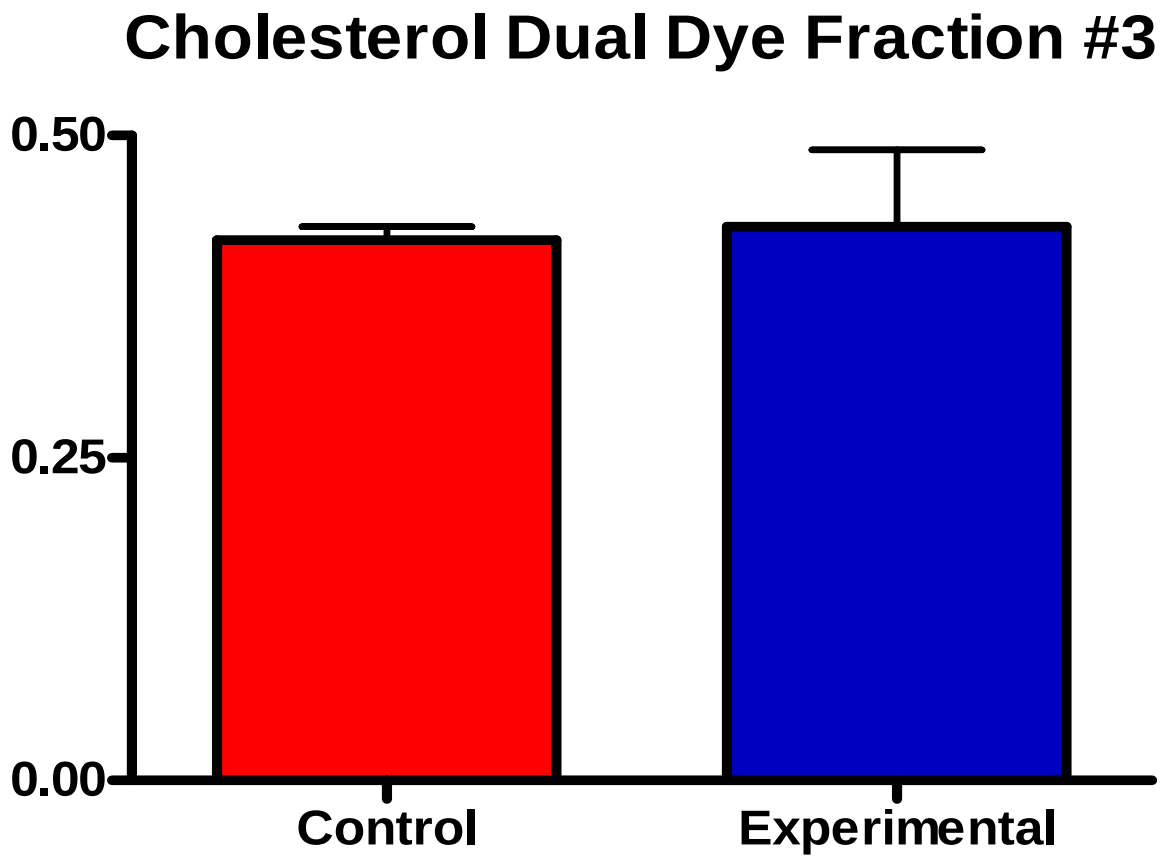
Liposome Formation Protocol

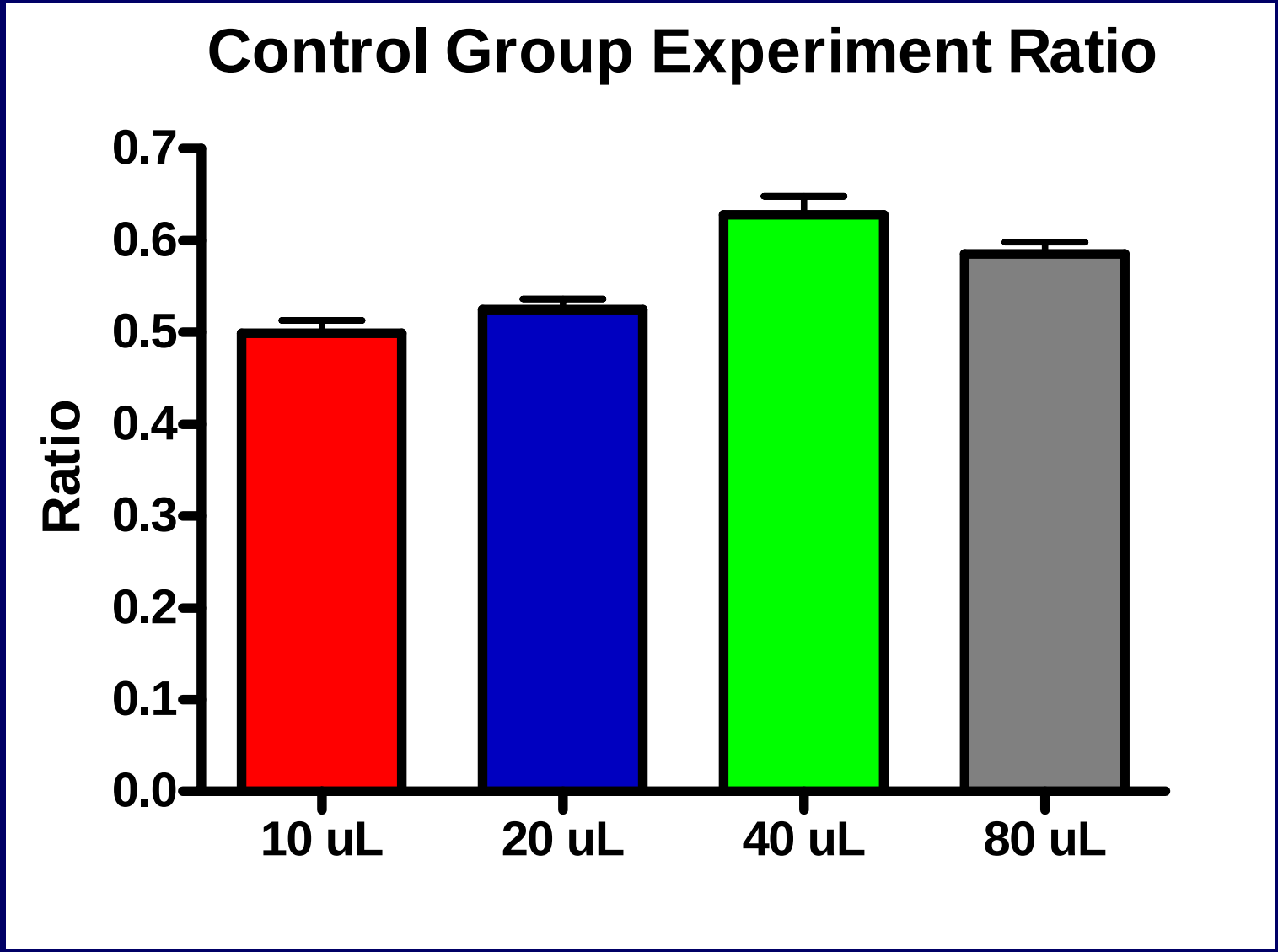
- For Alcohol and Organic Solvent Experiments
  - Add 10 µL Ratiometric Dye(2.5 mM) to DLPC(200 µL) (Avanti Labs)
  - Load Ratiometric Dye/DLPC onto Platinum electrodes in electroformation chamber
  - Form liposomes under AC electric field in sucrose solution- 12 mL
  - Extract liposome suspension
  - Add 500ul of liposome suspension to 500ul of stock sucrose solution

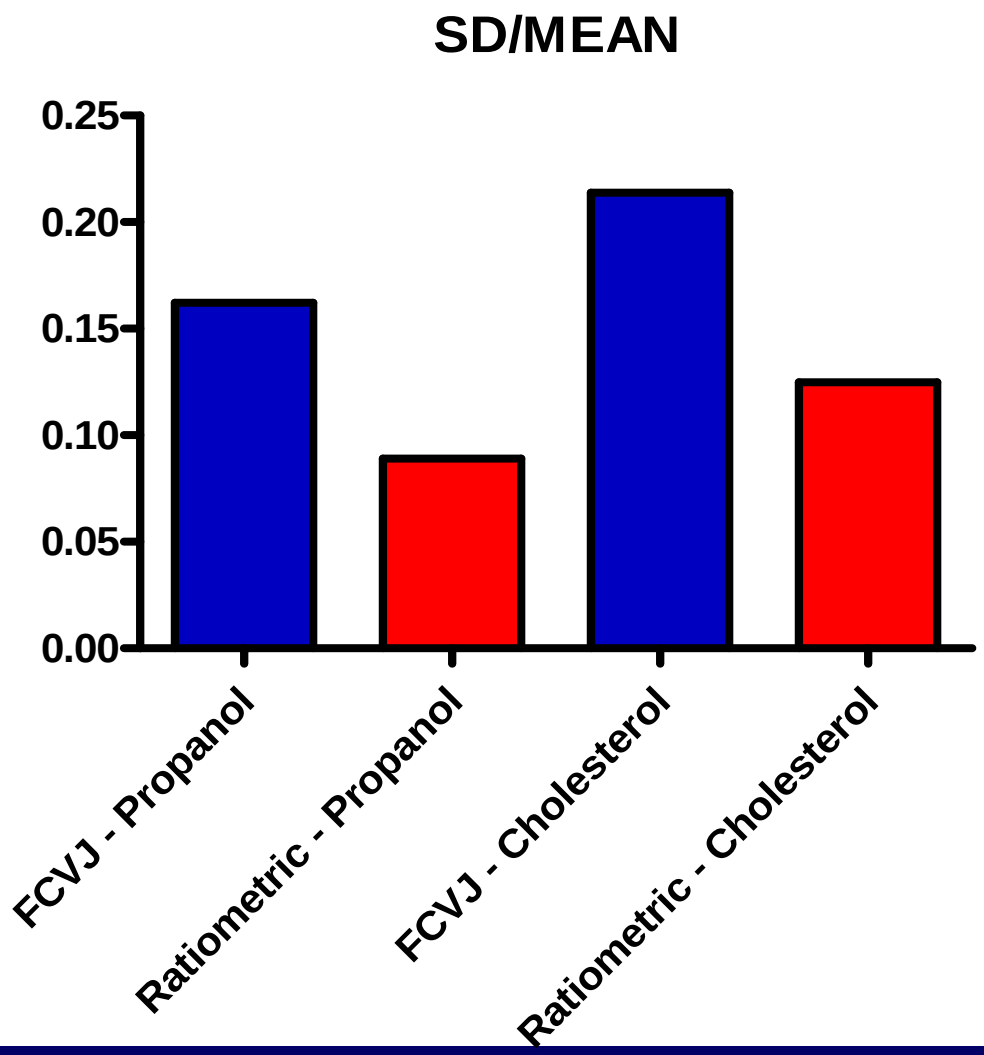
Excitation Parameters

- Coumarin Excitation
  - Excitation Wavelength=340nm
  - Scan from 370-420
- Rotor Excitation
  - Excitation Wavelength=430
  - Scan 450-520
- All experiments conducted at 22°C
- 10 minute equilibration time


Dual Dye Fraction #3 Ratiometric Propanol (DLPC)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control
Experimental - Propanol


Cholesterol Dual Dye Fraction #3
0.50
0.25
0.00
Control
Experimental


Control Group Experiment Ratio
Ratio
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10 uL
20 uL
40 uL
80 uL


SD/MEAN
0.25
0.20
0.15
0.10
0.05
0.00
FCVJ - Propanol
Ratiometric - Propanol
FCVJ - Cholesterol
Ratiometric - Cholesterol

Conclusion

- Ratiometric Dye incorporated into membranes
- Rotor was sensitive to changes in membrane viscosity
- Reduction in error significant
- Effective tool for measuring membrane viscosity changes

Future Work

- Continue to optimize rotor/reference structure
- Integrate with fluoroviscometer technology
- Incorporate ratiometric dye within cell membranes